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The recirculating lymphocyte pool of the rat: a systematic description of the migratory behaviour of recirculating lymphocytes

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Summary. A comprehensive study of lymphocyte traffic in AO rats was performed under conditions as near to the physiological state as was practicable. In the light of previous results on the effect of environmental factors on the migratory behaviour of lymphocytes, thoracic duct cells were passaged from blood to lymph in an intermediate rat before injection into a series of recipients for examination at time intervals from 1 min to 24 hr. At 1, 2 and 5 min after injection most of the labelled cells were in the blood, lungs and liver. The concentrations in these compartments fell over the next 25 min as the cells entered the spleen, lymph nodes (LN) and Peyer's patches according to a regular pattern. The peak localization in these latter organs occurred between 1 hr and 18 hr. Each organ had a characteristic time pattern of lymphocyte localization. Entry into mesenteric LN lagged behind other LN until 2·5 hr after injection following which mesenteric LN localization easily outstripped that in other LN to reach a delayed peak at 18 hr. Intravenously injected lymphocytes began to reappear in thoracic duct lymph in large numbers earlier than in previous studies so that the time taken for most T lymphocytes to cross LN from blood to lymph fell within the broad time band of 4–18 hr. Lymphocytes took on average 5–10 min to cross high endothelial venules when entering LN from the blood.

Abbreviations: TDL, thoracic duct lymphocytes; LN, lymph node(s); HEV, high endothelial venules.

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INTRODUCTION

The traffic of lymphocytes from the blood into the tissues has often been studied by tracing the fate of radioactively labelled lymphocytes after intravenous injection into syngeneic recipients. Our recent work (Ford & Smith, 1982; also unpublished data) has revealed two pitfalls in this technique which throw into question the quantitative reliability of previous kinetic studies of lymphocyte migration in experimental animals and man including data from this laboratory and from many others (reviewed by de Sousa, 1981). The two pitfalls are (i) the ability of thoracic duct lymphocytes (TDL) to cross high endothelial venules (HEV) was drastically impaired by collecting the cells overnight at 0°, and (ii) TDL re-isolated from the spleen or lymph nodes (LN) of a primary recipient were also impaired in their capacity to enter LN compared to TDL re-isolated from blood or lymph. This was a consequence of the microenvironment from which recirculating lymphocytes were isolated and presumably also applies to traffic experiments that start with

the preparation of cell suspensions from the spleen or LN for radioactive labelling and re-injection.

The aim of the present study was to record in detail the distribution and recirculation of lymphocytes under conditions as near as possible to the physiological situation. After they had been radioactively labelled *in vitro* TDL were passaged from blood to lymph through an intermediate. This manoeuvre afforded two advantages. First, only accredited recirculating lymphocytes were studied. Second, before injection into the final recipient the lymphocytes were kept *in vitro* for only a short time (*ca* 1 hr) and subjected to minimal handling.

The migration of these TDL (passaged, 1 hr, room temperature [RT]) was studied by injecting them i.v. into a series of recipients and examining three aspects of their distribution as follows—(i) a comprehensive survey of thirteen tissues at thirteen time intervals from 1 min to 24 hr after injection by whole organ counting of ^{51}Cr, (ii) the tempo of recirculation from blood to thoracic duct lymph by injecting TDL into cannulated recipients and taking 32×90 min fractions of lymph over the next 48 hr and (iii) the estimation of the time taken for lymphocytes to cross HEV into LN by autoradiography of LN removed at four time intervals after injection. Counting labelled cells in different anatomical compartments of the LN permitted estimates of the transit time across HEV.

MATERIALS AND METHODS

Rats

AO rats were used throughout the study. The donors of TDL and the intermediates used for passage from blood to lymph were adult males; recipients were young adult females of body weight averaging 168 g (Table 1).

Thoracic duct cells

The cannulation of donors of thoracic duct lymphocytes, the conditions of collection, labelling *in vitro* with sodium-[^{51}Cr] chromate and reinjection into a cannulated intermediate rat were all performed as described by Smith & Ford (1983a). Labelling *in vitro* with uridine-5-[^{3}H] (TRA.178, Amersham) and uridine-[^{14}C](U) (CFB.51, Amersham) was performed as described by Ford (1977).

Distribution in recipients

Recipients of i.v. injected lymphocytes were anaesthetized with ether before killing. A 2 ml sample of blood was taken from the inferior vena cava into a heparinized syringe and then the aorta was severed so that the blood pressure fell precipitously and presumably the exchange of lymphocytes between compartments ceased. With practice the time elapsing between the start of withdrawal of the blood sample and cessation of blood flow was reduced to 20–30 sec. Particular care was taken with the timing of samples from recipients killed at the early time intervals (1–10 min) but of necessity the blood sample was usually a few seconds before the nominal time and blood flow ceased a few seconds after. The rats killed at 1–5 min after injection were kept under light ether anaesthesia between injection and killing. At least five rats were examined at each time interval.

The following organs were removed from recipients of ^{51}Cr-labelled TDL for cleaning, weighing and radioactive counting as previously described—six superficial cervical LN, two to four deep cervical LN, two to three coeliac LN (Smith, Martin & Ford, 1980), the mesenteric LN chain excluding the ileocaecal LN, the right popliteal LN (stimulated 3 days previously with 0·10 ml of a 10% [v/v] suspension of sheep erythrocytes), the left popliteal LN (unstimulated), Peyer's patches, three samples of small intestines with Peyer's patches removed, the spleen, the left lung with the large hilar vessels removed, samples of liver (*ca* 1 g), the right tibia and the mononuclear cells in the 2 ml blood sample separated by ficoll/hypaque (see Table 1 for average weights).

The tempo of recirculation

The tempo of recirculation in final recipients was examined by collecting 90 min fractions of lymph from the injected rats over a 48 hr period. After centrifugation the radioactivity in each pellet was expressed as the % of the dose injected.

Autoradiography

LN from recipients of [^{3}H]-uridine-labelled TDL were removed, fixed and processed as previously described. After dipping in Ilford G5 emulsion they were developed and stained through the film with methyl green pyronin (Ford, 1977). Autoradiographs of LN sections were systematically scanned so that all labelled lymphocytes were attributed to an anatomical compartment (Fossum 1980; Bélisle & Sainte-Marie, 1981). Cells associated with HEVs were in the lumen, between endothelial cells or in the basement membrane.

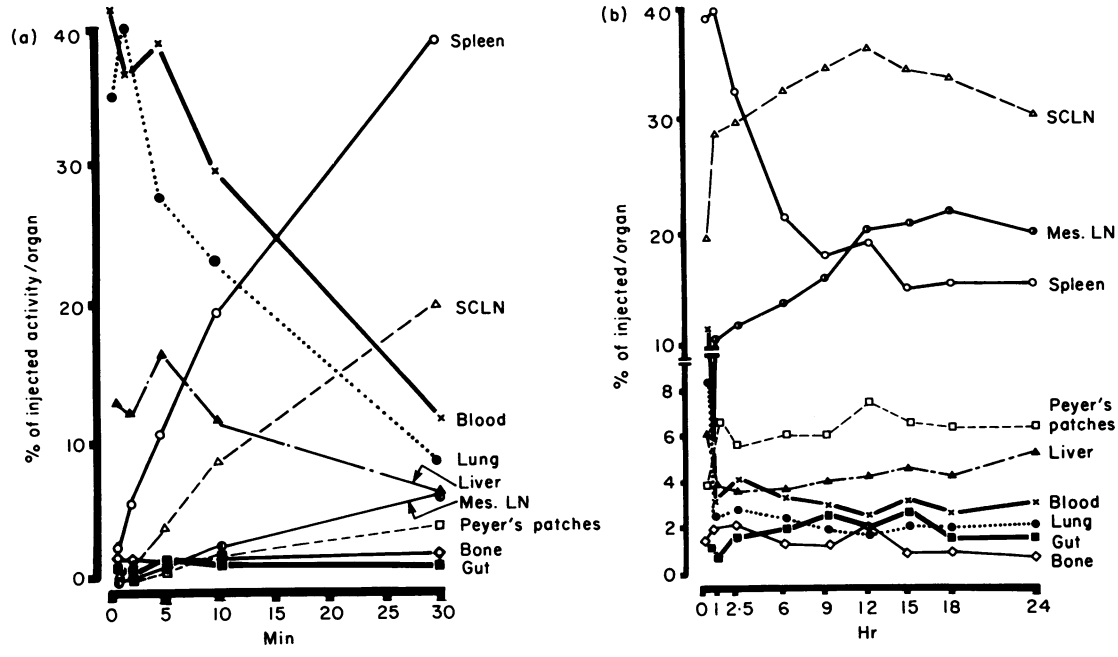

Figure 1. Localization of TDL (passaged, 1 hr, RT) after i.v. injection into syngeneic recipients as % of injected dose *per organ*. The compartments are labelled in the figure. Note that SCLN = subcutaneous LN in this figure. (a) Covers 0–30 min and (b) 30 min to 24 hr. There is a change in the vertical scale in (b) in order to separate compartments with a low localization of lymphocytes.

RESULTS

The organ distribution of [^{51}Cr]-TDL (passaged, 1 hr, RT)

The data presented in Figs 1 and 2 and Tables 1 and 2 provide a kinetic description of the distribution of lymphocytes after i.v. injection into a syngeneic recipient. For the first 6 hours after injection each time interval was two to three times longer than the previous interval. Although such times fit more evenly on to a log time scale nevertheless a linear time scale was preferred. For clarity of presentation the graphs are divided into 1–30 min and 30 min–24 hr phases, and lymphoid and non-lymphoid organs are graphed separately. The results of the scintillation counting data were calculated per organ and per gram of tissue and to some extent the data have been presented in both ways. As has been argued previously (Smith, Martin & Ford, 1980), each of these methods of presenting such results has special advantages. For example, when different LN are compared, such as the coeliac LN with the fifteen times larger superficial cervical LN, meaningful comparison requires consideration of the radioactivity per gram of LN (e.g. Table 2), but in order to decide whether an increase of localization in one compartment corresponds to a decrease in localization in another compartment, it is necessary to consider the localization per organ. The values derived from the data on radioactivity, organ weight and sample weight are expressed in Fig. 1 according to localization per organ and in Fig. 2 and Table 2 according to localization per gram of tissue.

Distribution from 1 to 5 min after injection. (Figs 1a, 2a, c.) At 1 min after injection approximately 40% of the injected dose was present in the lung, 40% in the blood, 13% in the liver and very little elsewhere. Considering that the cells probably had circulated through the lung only twice in that period and had circulated once or twice through other vascular beds, the extreme rapidity of uptake by the lung is remarkable. In the second minute after injection the lung localization rose slightly while the blood localization fell. Subsequently the lung localization moved in

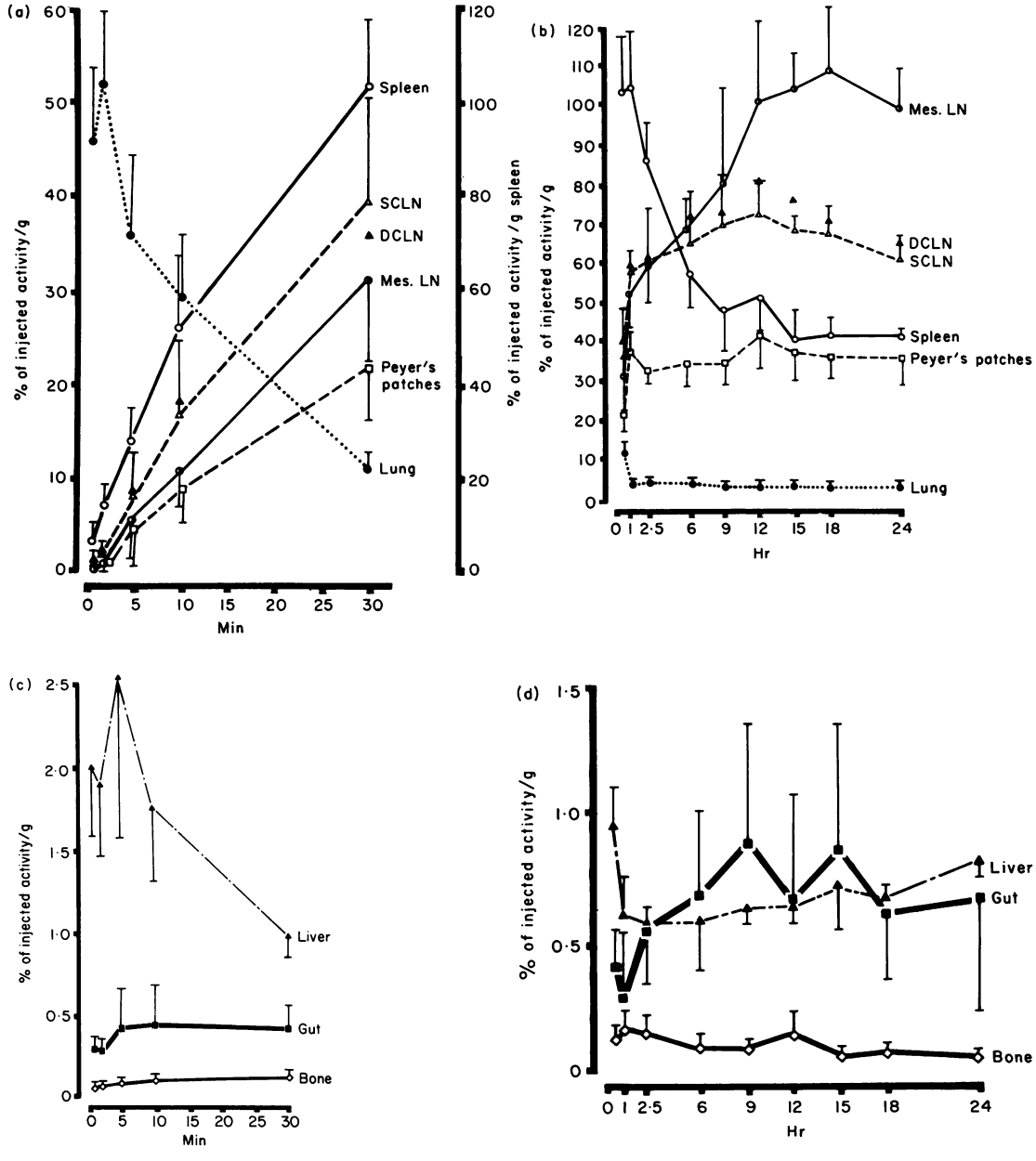

Figure 2. Localization of TDL (passaged, 1 hr, RT) after i.v. injection into syngeneic recipients expressed as % of injected dose *per g of tissue*. Note that SCLN = superficial cervical LN in this figure and DCLN = deep cervical LN. In (a) the spleen values are plotted on a reduced (× 2) vertical axis.

Table 1. Organ weights of 8–10 week female AO recipients

Organ	Weight g	SD	n
Superficial cervical LN	0·130	0·0297	65
Deep cervical LN	0·0606	0·0210	65
Coeliac LN	0·0081	0·0026	64
Mesenteric LN	0·158	0·0351	66
Peyer's patches	0·181	0·0127	10
Small intestine	3·01	0·321	10
Spleen	0·377	0·0545	66
Lung*	0·773	0·0966	10
Liver	6·42	0·822	10
R. popliteal LN (stimulated)	0·0113	0·0024	66
L. popliteal LN	0·0037	0·0011	66
Rats—body weight	167·8	17·3	

* Bronchi and major blood vessels cut out. Whole organs weighed from representative AO female rats.

parallel with the blood until the end of the study period at 24 hr. At 5 min after injection, the spleen localization had surpassed 10% of the injected dose and there was then significant radioactivity in all the LN and Peyer's patches. The spleen activity rose linearly with time from 1 to 10 min after injection and extrapolated back to zero at the moment of injection. Entry into LN seemed to be sluggish for the first 2 min after injection but because of low counts especially in the small LN the significance of this is uncertain.

Distribution from 5 min to 30 min after injection. (Figs 1a, 2a, c.) Throughout this distinct phase the radioactivity fell precipitously in the lungs and the blood and substantially in the liver. The radioactivity in the spleen, all the LN and Peyer's patches rose steadily from 5 min to 30 min maintaining the same relative position to each other (Fig. 2a). The values (% injected dose per g) for the two sets of cervical LN (deep and superficial) were identical to each other. Relative to the cervical LN, the spleen values were three times as high, the coeliac LN 1·27 times, the mesenteric LN 0·70 times and the Peyer's patches 0·55 times as great throughout this phase of steadily increasing localization. These systematic differences could not be explained by differences in blood flow either to the spleen or the different LN (Drayson, Smith & Ford, 1981, and unpublished observations by the methods described in that paper). Apparently this hierarchy of lymphoid organs reflects differences in the efficiency with which these organs can capture lymphocytes from the blood.

The pattern of the localization of lymphocytes in the gut and bone marrow while the blood concentration was falling steadily was more difficult to interpret. As

Table 2. Comparison of stimulated and unstimulated popliteal lymph nodes: localization of [^{51}Cr]-TDL in non-mesenteric lymph nodes

	Time after intravenous injection												
Lymph node	1 min	2 min	5 min	10 min	30 min	1 hr	2·5 hr	6 hr	9 hr	12 hr	15 hr	18 hr	24 hr
Right Popliteal (stimulated)				21·4 ±5·9	56·0 ±13·8	64·8 ±7·7	63·9 ±11·6	68·4 ±8·6	69·3 ±17·9	69·0 ±5·7	74·0 ±14·9	64·8 ±3·5	66·6 ±12·0
Left popliteal				15·7 ±5·8	42·5 ±15·6	56·3 ±11·0	61·8 ±15·0	58·8 ±16·9	74·8 ±21·5	87·9 ±17·5	75·5 ±13·6	68·1 ±6·4	63·1 ±9·4
Superficial cervical	1·4 ±0·4	1·8 ±0·8	7·9 ±5·2	16·9 ±7·8	39·7 ±9·1	57·4 ±5·9	59·3 ±14·7	64·8 ±13·2	69·0 ±13·2	72·3 ±8·7	68·3 ±3·3	67·3 ±7·6	60·2 ±11·8
Deep cervical	1·1 ±0·4	1·6 ±0·7	8·2 ±5·0	18·2 ±7·4	35·8 ±7·8	58·5 ±6·7	59·9 ±15·8	71·8 ±6·6	71·2 ±10·5	82·4 ±12·3	76·3 ±9·2	70·8 ±4·7	65·5 ±8·9
Coeliac			11·5 ±8·8	18·7 ±7·4	50·3 ±18·8	74·1 ±16·6	74·2 ±15·7	104·7 ±20·4	87·3 ±21·7	116·7 ±19·4	103·6 ±16·7	83·9 ±21·4	100·5 ±16·6
Ratio R. popl.LN/ L. popl.LN				1·41 ±0·23	1·40 ±0·32	1·17 ±0·13	1·05 ±0·15	1·23 ±0·32	1·16 ±0·19	0·83 ±0·14	0·99 ±0·18	0·96 ±0·09	1·06 ±0·21
No. of recipients	5	5	5	5	5	5	5	5	6	5	5	5	5

early as 1 min after injection, there was a significant localization in the bone marrow which doubled by 10 min to reach the shoulder of a plateau that was maintained for 2·5 hr. The gut localization also reached the shoulder of a plateau very early—5 min after injection. The finding of these plateaux for gut and bone marrow localization between 10 and 30 min when the blood concentration fell by a factor of three argued that the transfer of lymphocytes from the blood to these organs does not follow simple first-order kinetics as appears to be the case for the spleen and LN. Alternatively the preferential localization of minor subsets of TDL to the bone marrow or gut may explain these patterns.

Distribution from 30 min to 24 hr after injection. (Figs 1b, 2b, d.) The peak spleen localization was found at 60 min after i.v. injection but the increase between 30 and 60 min was small. There followed a sharp decline in splenic localization to about 50% of the peak level by 6 hr as has been found previously (Ford & Smith, 1979). The temporal pattern of LN localization was in great contrast to the spleen and there were highly significant differences between different LN and Peyer's patches. The latter reached a peak (or sharp shoulder) at 60 min following which there were only minor fluctuations up to 24 hr. The cervical LN and the coeliac LN reached a distinct shoulder at 60 min corresponding to the nadir in the blood but continued to increase steadily to reach a peak at 12 hr followed by a symmetrical fall from 12 to 24 hr (Fig. 1b). The mesenteric LN, which trailed behind the cervical LN from 5 to 30 min, overtook the cervical at 2·5 hr and continued to increase steeply to 12 hr and then less steeply to an 18 hr peak, at which time the mesenteric LN localization per gram was 63% greater than the cervical LN localization. Another way of expressing this difference in the temporal pattern is that between 1 and 18 hr after injection the mesenteric LN localization more than doubled while the cervical LN localization increased by only 19%.

Antigenic stimulation of the popliteal LN 3 days previously had increased its weight threefold (Table 1); the effect on lymphocyte localization can be inferred from Table 2. At 10 min and 30 min after the injection of labelled lymphocytes, their localization in the stimulated popliteal LN was 40% higher than in the contralateral LN on a per gram basis (second bottom row of Table 2). However, at 1 hr and later times there was little difference between the two popliteal LN and the values for other non-mesenteric LN were also similar in accordance with Drayson, Smith & Ford (1981). The tentative interpretation is that even on a per gram basis the enlarged LN was more efficient at capturing lymphocytes from the blood and lymphocytes may begin to leave the stimulated LN slightly earlier. It is noteworthy that there was no evidence of prolonged retention of lymphocytes at this stage after antigen stimulation, although application of the method described by Zatz (1976) would have indicated lymphocyte 'trapping' simply because of enlargement of the LN.

Since there were only minor differences in the temporal localization between the quiescent left popliteal LN and the heavily stimulated right popliteal LN, the pronounced difference between the mesenteric LN and other LN could not be explained in terms of the intensity of antigenic stimulation. It is rather implausible that the HEV of the mesenteric LN, after an early period during which they are less effective than HEV in other LN, could become more efficient in capturing lymphocytes between 2·5 and 18 hr after injection. The 'excess' of lymphocytes appearing late in the mesenteric LN seems likely to be due to cells reaching them via afferent lymphatics after leaving blood vessels in the wall of the gut and in Peyer's patches. Between 0·5 hr and 9 hr after injection, the 'excess' localization in the mesenteric LN amounted to 5·1% of the injected dose as calculated by extrapolating the 5, 10 and 30 min values for the mesenteric LN assuming a constant ratio to the cervical LN values (0·70). The high flux of cells recorded in afferent intestinal lymph (Gowans & Steer, 1980) is consistent with this notion that cell influx via the afferent lymphatic is a major route of entry into mesenteric LN.

Tempo of recirculation from blood to lymph

The transit time of lymphocytes travelling across LN from blood to thoracic duct lymph has been estimated by collecting timed fractions of lymph after the i.v. injection of radioactive lymphocytes (e.g. Ford & Simmonds, 1972). Since previous estimates of 12–18 hr for the modal transit time must have been affected by the impaired entry of lymphocytes into LN as a result of the conditions of collection, the transit time of TDL (passaged, 4 hr, RT) was estimated by injection into cannulated rats from which 1·5 hr fractions of lymph were collected for estimation of cell associated radioactivity. For comparison similar experiments were performed with TDL collected and labelled under 'standard' conditions (16 hr, 0°, non-passaged).

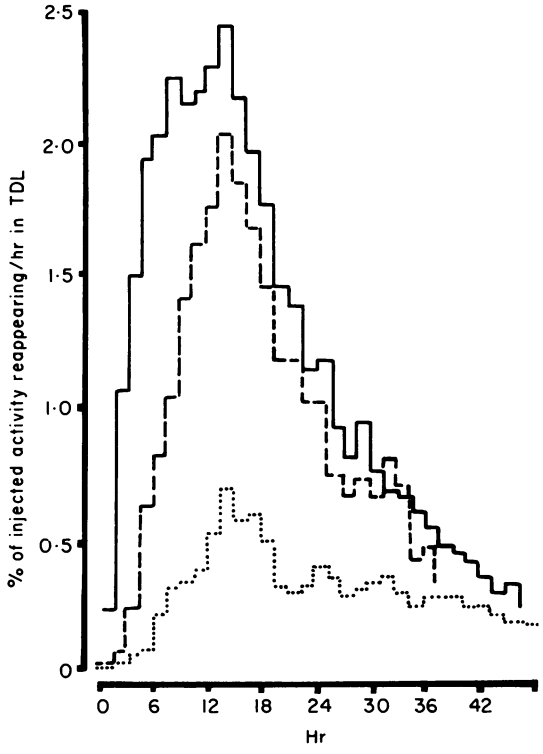

Figure 3. The temporal pattern of lymphocytes recirculating from blood to thoracic duct lymph. Fractions were collected every 1·5 hr but the recoveries are plotted per hour. (——) TDL (passaged, 4 hr, RT); (– – –) TDL (passaged, 16 hr, 0°; (· · · · ·) TDL (non-passaged; 16 hr, 0°, then labelled *in vitro*).

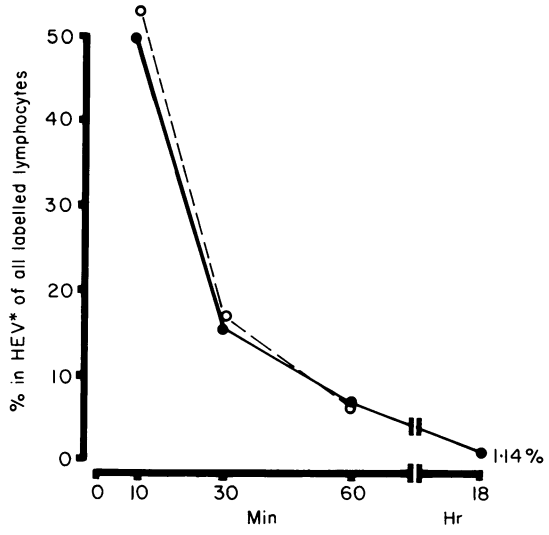

Figure 4. Autoradiographic study of the location of i.v. injected lymphocytes in LN sections. (●——●) TDL (passaged, 4 hr, RT); (O– – –O) TDL (non-passaged, 16 hr, 0°, then labelled *in vitro*). *'In HEV' means in the lumen, or between endothelial cells, or within the basement membrane. The great majority of lymphocytes not associated with HEV were in the extravascular compartments of the cortex or medulla (Table 3).

The TDL were labelled alternatively with [^{3}H]-uridine or [^{14}C]-uridine so that the recovery patterns of two populations could be recorded in one recipient (Ford & Simmonds, 1972). A third set of TDL was labelled *in vitro*, passaged from blood to lymph and collected at 0° for 16 hr (Fig. 3).

As anticipated there were large differences in the temporal patterns of reappearance in lymph between these three populations of TDL. While the pattern for TDL (16 hr, 0°, non-passaged) was similar to published results (Ford & Simmonds, 1972), with a peak recovery around 15–18 hr after injection and very few cells appearing in the first 6 hr, the recovery of TDL transferred near to physiological conditions (passaged, 4 hr, RT) was dramatically greater at early time intervals. For example, in the first 6 hr after injection, the cumulative recoveries were respectively 0·29% and 6·4% of the injected dose. This relative difference gradually diminished so that over the first 24 hr the cumulative recoveries were respectively 12·1% and 39·8% of the injected dose. Large numbers of TDL (passaged, 4 hr, RT) were recovered between 4·5 hr and 19·5 hr after injection with the peak recovery varying from 8 hr to 15 hr in individual experiments. Therefore it is difficult to give a revised estimate of the modal transit time of lymphocytes across LN but the plot of TDL (passaged, 4 hr, RT; Fig. 3) is nearly symmetrical about a 12 hr vertical axis if allowance is made for skewing to the right because of the delayed appearance of cells that have travelled from spleen to LN to lymph and cervical LN to LN to lymph. Thus the best estimate for an 'average' transit time of T lymphocytes to cross LN is approximately 12 hr.

Rate of crossing high endothelial venules estimated by autoradiography.

TDL were labelled *in vitro* with [^{3}H]-uridine (Ford, 1977) passaged from blood to lymph in an intermediate rat that had been subjected to 500 rads of whole body irradiation from a linear accelerator 6 days previously and collected for 3–4 h at RT. They were

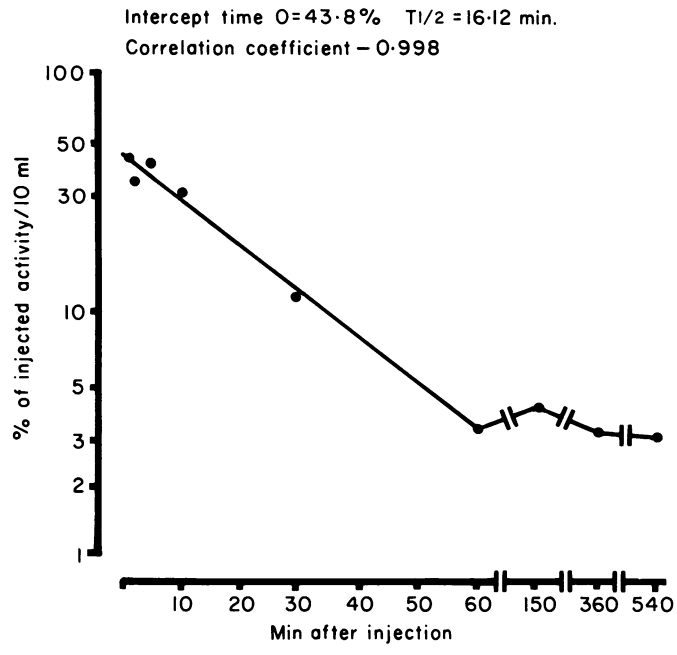

Figure 5. Blood concentration of labelled lymphocytes for 9 hr after i.v. injection of TDL (passaged, 1 hr, RT) plotted on a semi-logarithmetic scale. Note the strictly exponential fall in blood concentration up to and including 60 min after injection. This phase of exponential disappearance was sharply broken by cells returning to the blood.

injected i.v. into a series of recipients which were killed precisely 10 min, 30 min, 60 min and 18 hr later. The proportion of labelled lymphocytes associated with HEV in mesenteric and cervical LN is graphed in Fig. 4 which also includes similar data at 10, 30 and 60 min following the injection of TDL collected for 16 hr at 0° and injected without passage from blood to lymph. Table 3 provides more detailed information of the precise location of labelled lymphocytes (passaged, 4 hr, RT) at 18 hr after injection.

Remarkably about 50% of lymphocytes had already crossed HEV by 10 min after injection as was found in an independent study (Cox & Ford, 1982). Extremely few ($\leqslant 0{\cdot}2\%$) were seen in association with other small blood vessels at any time. The proportion of lymphocytes associated with HEV had diminished to 15–16% at 30 min and continued to decrease to 1·14% by 18 hr. The plots for the passaged (4 hr, RT) TDL and the non-passaged (16 hr, 0°) TDL were almost superimposed although smaller numbers of the latter were found in the whole LN as expected. It was concluded that the functional impairment following maintainance *in vitro* is extraordinarily selective; it only affects the initial stage of adhesion to HEV and the minority of TDL (16 hr, 0°) that do adhere can cross the HEV as quickly as do TDL (4 hr, RT).

Table 3. Autoradiographic estimate of the location of labelled lymphocytes (passaged, 4 hr, RT) in the mesenteric and cervical lymph nodes 18 hr after injection

Compartment	% of all the labelled lymphocytes in the sections
High endothelial venules (in lumen, wall or basement membrane)	1·14
Cortex (deep and superficial but excluding germinal centres)	91·1
Medulla (sinuses and cords)	6·8
Subcapsular sinus	1·0
Germinal centres	0
Associated with other blood vessels	0

The time taken for lymphocytes to cross HEV can be roughly estimated in two ways. The observation that about 50% of the labelled cells have crossed HEV by 10 min after injection suggests that the average transit across HEV is about 10 min, since the blood

concentration is maximal immediately after injection. However, the blood concentration remains very high throughout the first 5 min and the LN localization does not begin to rise much until after 2 min (Fig. 1a). Thus the average transit time across HEV may be rather less than 10 min.

The second way is to consider the distribution of labelled lymphocytes within LN at 18 hr after injection. The blood concentration has been steady at about 3% of the injected dose for many hours previously and the injected TDL were approaching equilibrium with the recipients' recirculating pool. Under steady state conditions the ratio of labelled lymphocytes in HEV to labelled lymphocytes in the entire LN will be the same as the ratio of average transit time across HEV to average transit time across the whole LN from blood to lymph. If the average LN transit time is taken as 12 hr from Fig. 3, then the HEV transit is $12 \times 60 \text{ min} \times 1{\cdot}14\% = 8$ min. The concordance of these two methods of estimation suggest that most lymphocytes take 5–10 min to cross HEV from the blood to beyond the basement membrane.

DISCUSSION

The relevance of lymphocyte recirculation to the generation of immune responses has already been argued sufficiently (Gowans & McGregor, 1965; Ford, 1975; Parrott & Wilkinson, 1981). A second reason for aiming at a thorough understanding of the recirculating lymphocyte pool is that it may be useful as a paradigm for investigating the selective migration from the blood of other cells, especially polymorphs and monocytes. The primary purpose of this paper is to provide systematic data on lymphocyte traffic in the young adult female AO rat as a background to quantitative studies of immune responses *in vivo* (Bell, 1981). A certain amount of interpretation was added to the results as they were presented in the previous section since this seemed appropriate.

An unusual aspect of this study has been the inclusion of five time intervals between 1 min and 30 min after injection. This turned out to be particularly rewarding because the early pattern of localization was easily interpretable. The numbers in the spleen, LN and Peyer's patches climbed steadily as the numbers declined exponentially in the bood and lungs (Figs. 1a, 2a, 5). The rate at which lymphocytes enter these compartments appears to be directly proportional to their concentration in the blood as was suggested by isolated organ experiments (Ford, 1969a; Sedgley & Ford, 1976).

There are few detailed studies of lymphocyte distribution at such early time intervals. Schnuda (1978) labelled blood lymphocytes with [^{3}H]-uridine before injection into syngeneic rats. He found high counts in the para-aortic LN after 1 min which fell to a nadir after 3 min, then rose to a 30 min peak. There was a delay of 5 min before entry into the spleen began. His conclusion was that 'small blood lymphocytes recirculate continuously between peripheral blood and the lymph node with a duration less than 3 min per cycle'. The distribution of recirculating thoracic duct lymphocytes early after injection was not remotely similar but there is a formal possiblity that Schnuda's observations can be explained by a population of cells in blood that do not recirculate into lymph. However, a human study by Wagstaff, Gibson, Thatcher & Crowther (1982) on the early localization of autologously reinfused blood lymphocytes did not suggest either a very rapid entry into LN or a delay in the entry into the spleen.

The next part of the discussion covers our interpretation of lymphocyte traffic in different organs.

The blood

Following a very rapid exchange with the lung within the first 1–2 min after injection the blood level fell precisely exponentially for the next 60 min with a 'half-life' of lymphocytes in the blood of 16 min (Fig. 5). The exponential fall stopped abruptly between 1 and 2·5 hr after injection because cells had started to return to the blood especially from the spleen. The blood level at 2·5 h was slightly above the 1 hr level but there was no marked overshoot. From 6 hr onwards the blood level was at a nearly steady state at approximately 3% of the injected dose.

The lungs

Between 5 min and 24 hr after injection there was very close correspondence between the numbers of injected lymphocytes in the blood and in the lungs. It is only in the first 5 min after injection that the blood and lung values fluctuated in opposite directions which is compatible with a rapid exchange. A current electron microscopic autoradiographic study of the precise site of [^{3}H]-uridine-labelled lymphocytes in the lung 10 min after injection shows that they are almost all tightly surrounded by capillary endothelium (unpub-

lished observations of Rattray & Ford). In conclusion there is an intravascular pool of lymphocytes in the lung that exchanges with the central blood pool within 1 min or less.

The liver

The high localization at 1–10 min after injection and the fall to a minimum of 3–4% of the injected dose by 1–2·5 hr suggested a similarity to the pattern of localization in the lung. At 10 min after injection, most labelled lymphocytes in the liver were intravascular as observed by autoradiography (Rattray & Ford, unpublished). Apparently there is a pool of intravascular lymphocytes in the liver that exchanges with the central blood pool rather more slowly than the exchange between the blood and the lung. Although damaged lymphocytes certainly localize in the liver (Bradfield & Born, 1973) and there is a small traffic of lymphocytes through into hepatic lymph (Smith, McIntosh & Morris, 1970), the high early localization of passaged TDL is more likely to represent transient intravascular pooling.

The spleen

Approximately 40% of the injected lymphocytes were present in the spleen at 30 and 60 min after injection. Splenic localization fell precipitously between 1 hr and 6 hr after injection, although the steady state value of 15% of the injected dose was not reached until 15 hr after injection. As the localization in the spleen was falling from the peak, it passed the halfway mark to the steady state value at just over 4 hr by interpolation. This was in accord with a previous estimate for the modal transit time through the spleen of 4–5 hr (Ford, 1969a, 1969b), accepting the extensive evidence that the falling radioactivity in the spleen reflected the release of recirculating lymphocytes (Bradfield & Born, 1973). Non-passaged TDL (16 hr, 0°) entered the spleen in equal numbers and equally as fast, but left it rather more slowly (Smith *et al.*, 1980) in comparison with passaged TDL (1 hr, RT).

Lymph nodes and Peyer's patches

Previous time-course studies of the accumulation of lymphocytes in LN have suggested a gradual, nearly linear build up over about 18–24 hr (e.g. Smith *et al.*, 1980). It is now apparent that this pattern was distorted by the impaired ability of lymphocytes held *in vitro* to enter LN early after injection. A novel finding of the present experiments is that the localization of lymphocytes in all LN and Peyer's patches climbed very rapidly throughout the first 60 min after injection, to reach a distinct shoulder corresponding to the nadir in the blood concentration. The sharpest shoulder was in the case of Peyer's patches where the radioactivity fluctuated only slightly between 1 hr and 24 hr, presumably because lymphocytes began to leave Peyer's patches early after injection. A striking difference in the time pattern of localization was observed between the mesenteric LN and subcutaneous LN from 1 hr onwards (Fig. 2b). At 2·5 hr after injection, the mesenteric LN overtook the cervical and popliteal LN after lagging behind at all earlier time points. As already argued, the exceptional increase in mesenteric LN radioactivity between 1 h and 18 h of injection was probably attributable to cells that had left the blood in the wall of the intestine and to a lesser extent in Peyer's patches. The mesenteric LN may be unique in that they receive a substantial proportion of their incoming lymphocytes (perhaps 30–40%) via afferent lymphatics rather than directly across their HEV.

The small intestine

The pattern of lymphocyte localization differed from that in other compartments in that a small but steadily increasing localization from 5 to 30 min after injection was followed by a dip or plateau and then a second rise from 2·5 hr to a peak at 9–15 h after injection, corresponding to 2·7% of the injected dose in the whole small intestine. One uncertainty is the contribution of lymphoblasts in the inoculum. Although thoracic-duct lymphoblasts migrate efficiently into the small intestine, most of the blasts in the TDL from the original donor would have been 'filtered out' in the intermediate because such cells (i) recirculate poorly, (ii) tend to appear earlier in the thoracic duct than the period of collection from the intermediate (16–24 h after injection; Smith *et al.*, 1980).

Small lymphocytes have been found in substantial numbers in the fine lymphatics draining the intestine (Gowans & Steer, 1980). It seems likely that some of the radioactivity in the intestine, especially early after injection, is attributable to a transient localization of small lymphocytes en route from the blood to the mesenteric LN.

The bone marrow

Entry into the bone marrow began at 1 min, increased to an early peak at 1–2·5 hr and then fell substantially as described by Rannie & Donald (1977). However, the proportion of passaged TDL (1 hr, RT) localizing

in the bone marrow was very much less than that found with non-passaged TDL (16 hr, 0°). The entry of lymphocytes into the bone marrow is quite modest, about 2–4% of all the cells leaving the blood. However, corticosteroids or adverse conditions of collection can greatly increase the bone marrow localization (Moorhead & Claman, 1972; Cox & Ford, 1982).

Conclusion

The tempo of recirculation from blood to lymph was faster than previously estimated, with an appreciable recovery of lymphocytes within 6 hr of injection and an 'average' transit time across LN estimated at 12 hr, of which only 5–10 min was spent transversing the walls of HEV. A puzzle of long standing was solved by recovering an average 39·8% of injected radioactivity from the thoracic duct population over the 24 hr after injection. It has been difficult to reconcile a recovery of only 15–20% of injected radioactivity over 24 hr with the rate at which thoracic duct output fell during chronic thoracic duct drainage (Gowans, 1957; McGregor & Gowans, 1963; Ford, 1968). Each study recorded an exponential fall between 1 and 4 days after cannulation with a halving time of 1·4 days, 1·6 days and 1·4 days respectively corresponding to a daily loss of 38·3% (average) of the recirculating pool. The revised estimate of the physiological rate of recirculation from blood to thoracic duct lymph fits well with the rate of depletion during thoracic duct drainage and adds confidence to the quantitative reliability of the present data. (Precise agreement between these two values could not be expected for at least two reasons: [i] gradual loss of radioactivity from cells; and [ii] though immediately after the injection of labelled cells they are all in the blood, at the start of thoracic duct drainage recirculating lymphocytes are widely distributed.)

An analogue computer analysis of data on the kinetics of lymphocyte recirculation in the rat spleen (Hammond, 1975) permitted a fuller interpretation than was offered in the original paper (Ford, 1969a). It seems likely that a more sophisticated analysis of the present data set would be equally fruitful. The data presented graphically are available in digital form on request.

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